

From past to future: suppressor mutations in yeast genes encoding translation termination factors

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Abstract

The study of the *SUP45* and *SUP35* genes of yeast *Saccharomyces cerevisiae* in the laboratory of Physiological Genetics of St. Petersburg State University began in 1964 when the first omnipotent nonsense suppressor mutations were obtained. During the following 55 years, a lot of information about these genes has been gained through the research efforts of various laboratories. Now we know that *SUP45* and *SUP35* encode translation termination factors eRF1 and eRF3, respectively. Both genes are essential, and *sup45* and *sup35* mutations lead not only to impaired translation but also to multiple pleiotropic effects. The aim of this review is to summarize known data about suppressor mutations in *SUP45* or *SUP35* genes.

Keywords: translation termination, suppression, *SUP45*, *SUP35*, eRF1, eRF3, nonsense mutations, missense mutations, [PSI⁺] prion, *S. cerevisiae*.

Introduction

In eukaryotes, translation termination requires two eukaryotic release factors: eRF1 and eRF3. The eRF1 protein belongs to class 1 translation termination factors, responsible for the recognition of the stop codon and peptidyl-tRNA hydrolysis, and eRF3 — to class 2 termination factors, functioning to stimulate the work of class 1 factors due to its GTPase activity. Most eukaryotic organisms have a single class 1 translation termination factor called eRF1 (Frolova et al., 1994) that recognizes all three stop codons. In eukaryotic cells, class 2 translation termination factors are represented by eRF3 proteins (Stansfield et al., 1995a; Zhouravleva et al., 1995). Although all living organisms have a similar general translation termination mechanism, there are significant differences primarily between prokaryotes and eukaryotes (see review by Kisselev et al., 2003). During the last years several additional proteins participating in eukaryotic translation termination have been identified, including ABCE1 (Rli1 in *S. cerevisiae*), Dbp5, PABP, Hrp1, Pub1 and Upf proteins (see reviews by Tieg and Krebber, 2013; Schuller and Green, 2018).

It should be noted that the role of translation termination factors in the cell seems not to be limited to participation in translation termination, and these proteins are also likely to be involved in other stages of translation and different cellular processes.

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The main goal of this review is to summarize known data about suppressor mutations isolated in *SUP35* or *SUP45* genes with an emphasis on results obtained in the Department of Genetics and Biotechnology of St. Petersburg State University.

1. The history of the discovery of the *SUP45* and *SUP35* genes

The study of the *SUP35* (*SUP2*) gene and functionally linked *SUP45* (*SUP1*) gene in *S. cerevisiae* has a history of more than fifty years. In 1964, the mutations in these genes, named *s1* and *s2*, recessive omnipotent nonsense suppressors, were obtained in the laboratory of Physiological Genetics of St. Petersburg State University (Inge-Vechtomov, 1964). The mutations in these genes caused the suppression of nonsense codons of all three types (Inge-Vechtomov and Andrianova, 1970), or omnipotent suppression. Developing a specific selection system for recessive suppressors promoted their further study. Haploid strains carrying the *ade1-14* and *his7-1* mutations (later identified as nonsense mutations UGA and UAA, respectively) were shown to revert to prototrophy mostly due to mutations in the genes *s1* and *s2* (Inge-Vechtomov and Andrianova, 1972). A similar phenomenon was found with simultaneous reversions to prototrophy for histidine and lysine in double mutants *his7-1* (UAA) *lys2-87* (UGA) (Inge-Vechtomov et al., 1988).

Later, similar omnipotent suppressor mutations were described in other laboratories: *sup35 u sup45* (Hawthorne and Leupold, 1974), *supP* and *supQ* (Gerlach, 1975). After that, by using the allelism test, it was shown that *sup1=sup45=supQ*, and *sup2=sup35=supP* (see Sherman, 1982). When searching for genes affecting the translation fidelity, in the strain [*PSI*⁺], the weak omnipotent suppressors *sup36* and *sup47* were isolated, that also turned out to be *SUP35* и *SUP45*, alleles, respectively (Ono et al., 1984). Moreover, the *GST1* gene (Kikuchi et al., 1988), a mutation in which led to the cell cycle block at the G1-S phase, also turned out to be identical to the *SUP35* gene.

In the search for allosuppressor mutations that could increase the suppression efficiency of the weak dominant suppressor *SUQ5*, the *SAL3* and *SAL4* genes were found, which proved to be identical to the *SUP35* and *SUP45* genes (Crouzet and Tuite, 1987; Crouzet et al., 1988). Thus, various genetic approaches allowed identification of the genes, mutations in which led to recessive omnipotent suppression, allosuppression, cell cycle disorders, as well as numerous pleiotropic effects. These genes were commonly referred to as *SUP45* (for *sup1*) and *SUP35* (for *sup2*), with their identity being confirmed by their cloning and sequencing (Surguchev et al., 1983; Breining et al., 1984; Himmelfarb et al., 1985; Telkov et al., 1986; Breining and Piepersberg, 1986;

Crouzet and Tuite, 1987; Crouzet et al., 1988; Kushnirov et al., 1988; Wilson and Culbertson, 1988).

Genetic research data collected by the early 1990s led to a conclusion that the products of the *SUP45* and *SUP35* genes are involved in the control of translation fidelity, with this fact being confirmed by the analysis of biochemical data. *In vitro* ribosomes isolated from mutants *sup45* and *sup35* showed a high level of errors, with defective Sup45 and Sup35 proteins being associated with the 40S subunit of the ribosome (Eustice et al., 1986). Later studies confirmed this and showed that Sup45 and Sup35 proteins interact with monosomes and polysomes, this association being stronger than with the 40S subunit (Didichenko et al., 1991; Stansfield et al., 1992).

The cloning and sequencing of the *SUP45* and *SUP35* genes showed that they encode proteins of 49 and 77 kDa, respectively (Himmelfarb et al., 1985; Breining and Piepersberg, 1986; Kushnirov et al., 1988; Wilson and Culbertson, 1988), their size exceeding the size of known ribosomal proteins. Moreover, the *SUP45* gene was expressed at a rather low level, since *SUP45* mRNA was only 1/10 of the level of mRNA of the L3 ribosomal protein (Himmelfarb et al., 1985). While Sup45 protein did not reveal homology with any of the proteins known at that time (Breining and Piepersberg, 1986), the C-terminal domain of Sup35 protein was 37 % identical to the yeast elongation factor eEF1-A (Kushnirov et al., 1988; Wilson and Culbertson, 1988). Taken together, these data suggest that Sup45 and Sup35 proteins may be translational factors closely associated with the small 40S subunit of the ribosome (Himmelfarb et al., 1985; Kushnirov et al., 1988; Wilson and Culbertson, 1988).

The estimation of the relative content of Sup45 and Sup35 proteins over the ribosomal fraction showed that each of these proteins is present in a cell in a ratio of 1 to 20 ribosomes (Didichenko et al., 1991; Stansfield et al., 1992). Consequently, Sup45 and Sup35 were more likely to be the factors of initiation or termination, rather than translation elongation, since the latter are usually present in equal stoichiometric ratios with the ribosomes (see review by Stansfield and Tuite, 1994).

The available biochemical data served as evidence for Sup45 and Sup35 proteins participating in the termination process, rather than translation initiation. Thus, even earlier studies proved a thermosensitive mutation in the *SUP35* gene to cause the disruption of protein synthesis (Smirnov et al., 1974) and accumulation of 80S ribosomes associated with peptidyl-tRNA (Smirnov et al., 1976). The identification of various types of tRNA complex allowed one to suggest that the defect under study was associated with translation termination but not translation initiation, as in the latter case, tRNA^{Met} would be the main component of the peptidyl-tRNA complex (Surguchov et al., 1980). The omnipotent sup-

pressor phenotype of the *sup45* and *sup35* mutants also suggested defects in the translation termination process.

The deletion analysis of the corresponding gene provided further evidence of Sup35 protein involvement in termination. In the Sup35 protein, one can identify three domains bounded by methionines at 1, 124, and 254 amino acid (aa) positions (Figure 1A). These domains differ in a number of properties. The N-terminal domain (aa 1–123) is enriched in Q and N residues and contains oligopeptide repeats. The M-domain (aa 124–253) is enriched with charged amino acid residues. The C-terminal domain (aa 254–685) reveals homology with the elongation factor eEF1-A. The *SUP35* gene (similar to the *SUP45* gene) is an essential gene (see below). The presence of only the C-terminal domain in the cell proved to be sufficient to compensate for the deletion of this gene. At the same time, the *TEF1* gene, encoding eEF1-A, could not compensate for the deletion of the *SUP35* gene, indicating the difference in the functions of eEF1-A and Sup35 proteins during the translation process. An increase in the content of the C-terminal domain in the cell led to anti-suppression (Ter-Avanesyan et al., 1993), while the over-expression of the full-length *SUP35* gene (or its N-terminal domain) caused omnipotent suppression (Chernoff et al., 1988; Chernoff et al., 1993; Ter-Avanesyan et al., 1993; Derkatch et al., 1996). Later, the prion hypothesis enabled these results to be explained.

By the beginning of the 1990s, in yeast, two cytoplasmic allosuppressors were found, identified as [*PSI*⁺] (Cox, 1965) and [*ETA*⁺] (Liebman and All-Robyn, 1984). The [*PSI*⁺] factor increased the suppression of weak tRNA suppressors, such as *SUQ5* (Cox, 1965) and resulted in lethality in combination with *SUP11-o* and *SUP3-o* (Cox, 1971). The [*ETA*⁺] factor, in turn, resulted in lethality in combination with *sup35-2* and *sup45-2* mutations but did not affect the viability of the other *sup35* and *sup45* mutants under study (Liebman and All-Robyn, 1984; All-Robyn et al., 1990). Later, [*PSI*⁺] and [*ETA*⁺] were shown to be different forms of the same factor [*PSI*⁺] (Zhou et al., 1999). For a long time, the molecular nature of this factor remained inexplicable until it was shown that the [*PSI*⁺] factor is a product of the *SUP35* gene (Chernoff et al., 1993; Doel et al., 1994; Ter-Avanesyan et al., 1994). Not long after that, R. Wickner suggested that Sup35 protein might be a yeast prion (Wickner, 1994).

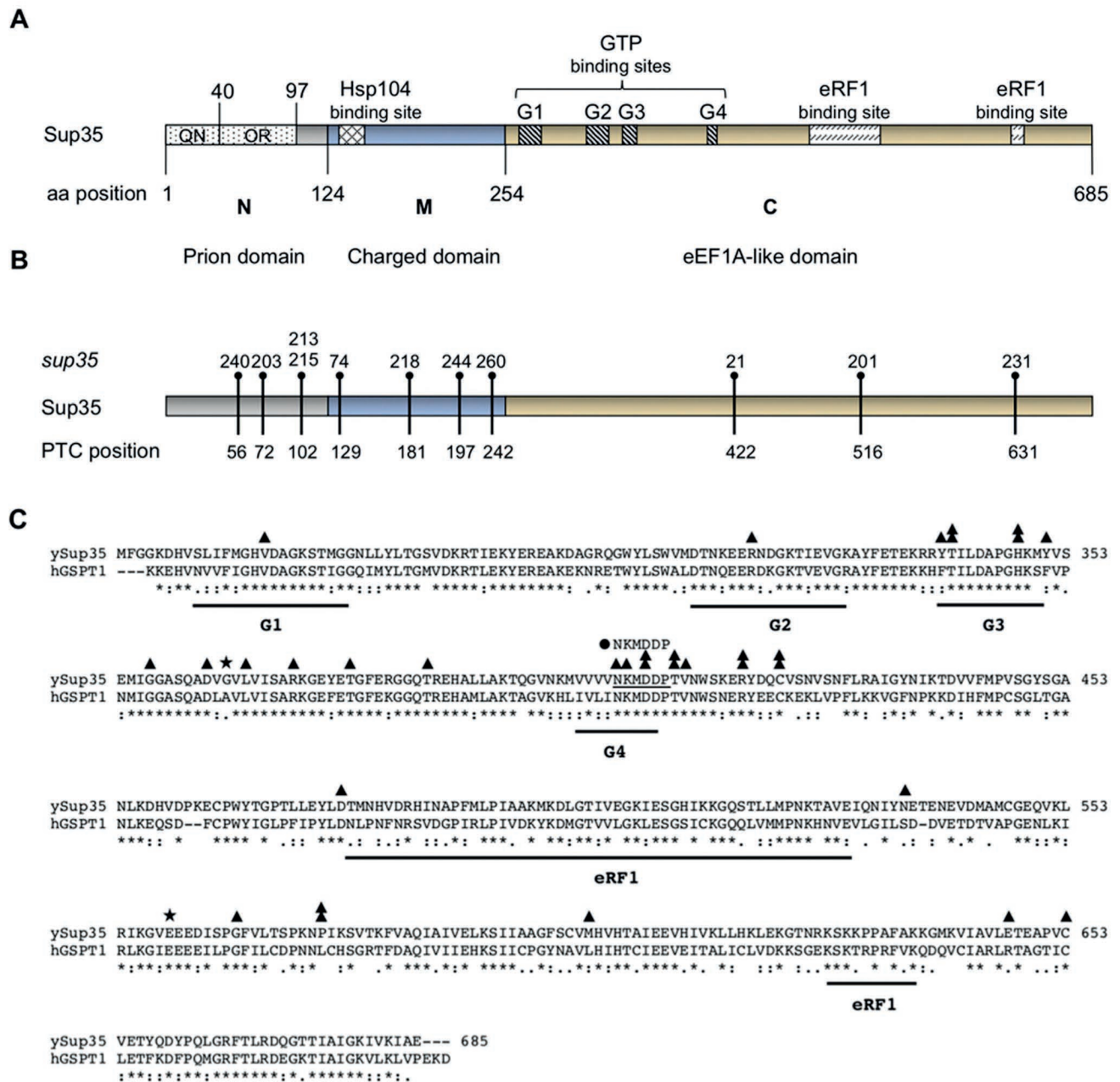
Thus, by the beginning of the 1990s, the exact role of Sup45 and Sup35 proteins in translation remained unidentified. Although in early works, Sup45 and Sup35 proteins were supposed to be involved in translation termination (Inge-Vechtomov and Andrianova, 1970), the lack of homology with prokaryotic release factors (RF), as well as the absence of an anti-suppressor effect during overexpression did not allow them to be

considered as translation factors (see Stansfield and Tuite, 1994). It was assumed that these proteins could play a supporting role in translation termination by interacting with release factors or with ribosomes. While prokaryotic translation release factors were first characterized a long time ago (Craigie et al., 1985; Craigie and Caskey, 1987), the eukaryotic termination factors remained unidentified for a long time. The cloning of cDNA encoding the RF of a rabbit was reported in 1990 (Lee et al., 1990). A surprisingly high degree of similarity between the protein and tryptophanyl-tRNA synthetase identified served as evidence that the identified gene encodes the rabbit tryptophanyl synthetase but not the RF factor (Frolova et al., 1993). Even in early experiments, translation termination in mammals was demonstrated to be a GTP-dependent process (Beaudet and Caskey, 1971; Konnecki et al., 1977), but to isolate a protein with GTPase activity was not possible.

In 1994, the laboratory of L. L. Kiselev in collaboration with some foreign laboratories found Sup45 protein to be a termination factor capable of recognizing stop codons (Frolova et al., 1994). Due to this property, it was named eRF1 and, together with the bacterial proteins RF1 and RF2, was assigned to class 1 termination factors. The following year, data was obtained that was significant to establish the role of Sup35 protein as a second termination factor; it was named eRF3 because its functions are analogous to the bacterial RF3 (Zhouravleva et al., 1995).

2. Molecular mechanism of the translation termination process in eukaryotes

In eukaryotes, for efficient translation termination, it is necessary that eRF1 should interact directly with eRF3 in the presence of GTP (Stansfield et al., 1995a; Zhouravleva et al., 1995; Frolova et al., 1996). It was shown that the interaction of the yeast eRF1 and eRF3 is possible only if eRF3 is associated with GTP (Kobayashi et al., 2004). This interaction is supposed to lead to conformational changes that allow eRF1 to interact efficiently with the stop codon in the A-site of the ribosome. Conformational rearrangements seem to be necessary for the exact correspondence of the distance between the GGQ motif and the “protein anticodon” in eRF1 (80 Å) and the distance between the peptidyl-transferase center of the ribosome and the stop codon (75 Å). However, the binding of the termination factors to the ribosome is not enough to effectively terminate translation. Additional conformational changes in the structure of eRF1 are required to correctly orient the GGQ motif in the peptidyl transferase center (Alkalaeva et al., 2006). The energy for such a rearrangement is provided by the hydrolysis of GTP by the eRF3. The eRF3 associated with GDP dissociates from eRF1 (Kobayashi et al., 2004). Given the above, the role of the eRF3 in transla-



tion termination was supposed to be reduced to a change in the conformational states of eRF1 (Alkalaeva et al., 2006; Fan-Minogue et al., 2008).

A distinctive feature of eRF3 is its ability to form a stable complex with eRF1 (Stansfield et al., 1995a; Zhouravleva et al., 1995; Frolova et al., 1998) due to the interaction between the C-terminal domains of both factors (Ito et al., 1998; Ebihara and Nakamura, 1999; Merkulova et al., 1999). It was assumed that eRF3 might play a role similar to bacterial RF3 by providing “recycling” (Zavialov et al., 2001). However, according to genetic data, the eRF3 GTPase activity is needed both to ensure correct recognition of stop codons by the eRF1 and for subsequent release of the newly synthesized peptide (Sallas-Marco and Bedwell, 2004). Taken together, the above data, as well as the translation termination simulation *in vitro*, made it possible to modify the existing translation termination model (Alkalaeva et al., 2006). eRF3 is believed to bind to the ribosome in the presence of GTP, and the subsequent hydrolysis of GTP is necessary for eRF1 to be able to induce the release of the newly synthesized polypeptide. The model given is consistent with the results of the crystal structure characteristics of the eRF3 (aa 196–662) C-terminal domain of *Schizosaccharomyces pombe* (Kong et al., 2004). Functional differences between eRF3 and RF3 may be due not only to their different origins but also to only one class 1 termination factor being present in eukaryotes, that should not only recognize the stop codons UAG, UAA, and UGA but also distinguish them from similar codons, especially the UGG codon (see reviews by Ehrenberg and Tenson, 2002; Kisselev et al., 2003; Nakamura and Ito, 2003). Such features of eukaryotic translation termination could require an additional step provided by eRF3.

3. Termination factor eRF3

3.1. STRUCTURE OF YEAST eRF3

The eRF3 proteins of most eukaryotes, except for some protozoa, have a three-domain structure (see review by Inge-Vechtomov et al., 2003). In *S. cerevisiae*, the eRF3 is encoded by the *SUP35* gene (Zhouravleva et al., 1995), which is an essential gene (Wilson and Culbertson, 1988; Grentzmann et al., 1994; Mikuni et al., 1994). The N-domain of yeast Sup35 was shown not to be necessary for the viability of the cell (Ter-Avanesyan et al., 1993). Interestingly, this domain (named also PFD — prion-forming domain) is responsible for the possibility of Sup35 prionization with the formation of the $[PSI^+]$ determinant (Ter-Avanesyan et al., 1994; Derkach et al., 1996; Paushkin et al., 1996). In the N-domain, two regions may be identified: a region rich in Q and N (within 1–40 amino acid residues) and a region of oligopeptide repeats (amino acid residues 41–97) containing five full

PQGGYQ(Q)QYN repeats from nine amino acids and one partial repeat, containing four amino acids PQGG (Kushnirov et al., 1988) (Fig. 1A). The N-domain of the eRF3 family proteins is highly variable, with its amino acid sequence differing even in closely related species.

The Sup35 M-domain is enriched with charged amino acids and is involved in maintaining prion in a series of cell divisions. A short section from 129–148 aa is involved in the interaction with Hsp104 and stimulates the ability of the chaperone to break down $[PSI^+]$ fibrils. Despite N- and M-domains being nonessential for Sup35 activity, their conservation suggests them to be a two-component functional unit, the critical role of which remains still unexplored. NM-domain forms reversible pH dependent biomolecular condensates (Franzmann et al., 2018).

The C-terminal domain of the Sup35 protein is necessary and sufficient to participate in translation termination (Ter-Avanesyan et al., 1993). It contains GTP-binding domains (Kushnirov et al., 1987; Hoshino et al., 1998) and an eRF1-binding site (Paushkin et al., 1997; Ito et al., 1998; Ebihara and Nakamura, 1999; Merkulova et al., 1999).

3.2. SUPPRESSOR MUTATIONS IN THE *SUP35* GENE

A dysfunction of Sup35 may account for a suppressor effect. Therefore, studying suppressor mutations may provide insight into the role of Sup35 in the translation termination process. When selecting mutations in the *SUP35* gene by simultaneous reversion of the *ade1-14* (TGA) and *his7-1* (TAA) mutations, 48 *sup35* mutations were obtained (Chabelskaya et al., 2004). After analyzing the content of Sup35 protein in these mutants, it was shown that in 32 of them, the amount of Sup35 did not differ from the strain carrying the wild-type *SUP35* allele, while a third of the strains (16 mutants) had a lower content of Sup35 protein compared with the original strain. 15 mutants were selected to be sequenced, with five having the same level of the full-length Sup35 protein as in the wild type and 10 revealing a reduced amount of the full-length Sup35 protein (Chabelskaya et al., 2004) (Table 1 and Fig. 1B, C). The molecular nature of these mutations and their comparison with those described in the literature is discussed in the following sections.

3.2.1. Nonsense mutations in the *SUP35* gene

Among ten *sup35* nonsense mutations characterized, seven are localized in the first third of the *SUP35* gene, encoding the NM-domain of eRF3 (aa 1–253) (Fig. 1B). Such an uneven distribution of nonsense mutations can be accounted for by an increased codon content, with a single substitution capable of resulting in a stop codon.

Table 1. The mutations in the *SUP35* gene, described in different works.
X means substitution of amino acid to stop codon; † indicates the lethality of mutation

Method of selection	Type of mutation	Nucleotide changes	Amino acid changes	Reference
UV-mutagenesis (strain SL183-21C)	Nonsense	T229A	L110X	Zhou et al., 1999
Suppression of <i>his7-1(UAA)</i> and <i>trp1-289 (UAG)</i> (strain 33G-D373)	Nonsense	C1264T	Q422X	Cosson et al., 2002
Suppression of <i>ade1-14(UGA) his7-1(UAA)</i> and <i>lys2-87 (UGA)</i> (strain 2V-P3982)	Missense	G1087A; C1133T; G1237A; C1724A	D363N; T378I; V413L; P575H	Volkov et al., 2002
Suppression of <i>ade1-14 (UGA)</i> (strain 74-D694)	Missense	G959T; A1052G; G1070A; G1256A; G1429T; A1607C; T1957C	R320I; Y351C; G357D; R419H; D477Y; N536T; C653R	Bradley et al., 2003
	Nonsense	C64T; C147G; C211T; C214T; G229T; G334T; G514T (two times); G553T; C629A; C668G; G1609	Q22X; Y49X; Q71X; Q72X; G77X; G112X; E172X (two times); E185X; S210X; S223X, E537X	
Suppression of <i>ade1-14(UGA)</i> and <i>his7-1(UAA)</i> (strain 1B-D1606)	Missense	C1115T; G1697A; G1808T; G1094T/G1675A;	R372K; G566N; M603K; G365V/ E559K	Chabelskaya et al., 2004
	Nonsense	C166T; C214T; A304T(two times); C388T; G541T; G586T; C724T; C1264T; A1546T; C1984T	Q56X; Q72X; K102X (two times); Q129X; E181X; E197X; Q242X; Q422X; K516X; Q631X	
	Duplication	18 bp duplication (begins at nucleotide 1217)	additional 6 aa (begins at aa 305)	
	Mutation in promoter	G(-183)T	no	Matveenko et al., 2019
Suppression of <i>lys9-A21 (UAA)</i> and <i>his7-1(UAA)</i> (strain 33G-D373)	Missense	A1234C; G1939A	T412P; E647K	Our unpublished data
	Nonsense	C388T	Q129X	
Site directed mutagenesis	Missense	T806G; A1043T; U/C1044G/A; A1999G; GAU1225UGG; A1255G	V269G†; H348L†; H348Q; K407E†; D409W†; R419G	Salas-Marco and Bedwell, 2004
PCR mutagenesis	Missense	A1219T; G1227A	N406I; D409N	Kobayashi et al., 2004
Suppression of <i>ade1-14(UGA) his7-1(UAA)</i> (strain SUP35C-2V-P3982)	Missense	C1157T; G1268A (two times); C1724A	T386I; C423Y (two times); P575H	Volkov et al., 2007
Suppression of <i>ade1-14(UGA) his7-1(UAA)</i> (strain pRSU1C-16AD1608)	Missense	T1018C; T1100C; C1235T/ C1236T	Y340H; L367S; T412I	
Site directed mutagenesis	Missense	A1021G; AC1021GA	T341A; T341D†	Fabret et al., 2008

The *SUP35* gene region encoding the NM-domain does contain 48 % of such codons compared with 33 % in the region encoding the C-domain of eRF3 and with 34 % in the entire yeast genome (Table 2). The absence of mutations resulting in the TGA stop codon among the *sup35-n* mutations can be explained by the reduced content of “potential” UGA codons (codons, single mutations which can lead to the appearance of the UGA codon). The *SUP35* gene sequence does contain 10 % of such codons, compared with 39 % and 51 % for potential codons UAG and UAA, respectively (Table 2). These data correlate with the number of isolated mutations containing TAG (4 mutations) and TAA (6 mutations) (Table 3).

It is worth noting that in the screening for Ade⁺ revertants twelve *sup35-n* mutations were isolated, with six being TAG mutations and the rest evenly distributed between TAA and TGA mutations (Bradley et al., 2003). The differences in the ratios of the types of *sup35-n* mutations can be explained by the differences in the mutant selection methods.

3.2.2. Missense mutations in the *SUP35* gene

The finding that all missense mutations isolated from different strains are localized in the C-terminal domain of eRF3 (aa 254-685) (Fig. 1C, Fig. 2 and Table 1) con-

Table 2. The distribution of potential stop codons in the *SUP35* and *SUP45* genes. The program “Countcodon” (<http://www.kazusa.or.jp/codon/countcodon.html>) was used to calculate the content of different codons

Gene	Domain (codons number)	The number of potential stop codons (%):			
		UAA	UAG	UGA	All
<i>SUP35</i>	N (123)	29.7	17.9	3.6	51.2
	M (139)	22.6	21.6	0.4	44.6
	C (433)	16.2	12.1	4.7	33.0
	N+M (252)	26.1	20.1	2.0	48.2
	N+M+C (685)	19.8 (51 %)	15.1 (39 %)	3.7 (10 %)	38.6 (100 %)
<i>SUP45</i>	N (132)	19.9	14.2	4.8	39.1
	M (120)	14.2	8.7	7.9	30.8
	C (185)	22.7	12.4	3.2	38.3
	N+M+C (437)	19.7 (53 %)	12.0 (33 %)	5.2 (14 %)	36.9 (100 %)
Total in the genome of <i>S. cerevisiae</i>		14.5 (43 %)	12.5 (37 %)	7.1 (20 %)	34.1 (100 %)

Table 3. Nucleotide context surrounding *sup45* and *sup35* nonsense mutations

Mutation	Amino acid position	Nucleotide context surrounding preliminary termination codon*: 5' stop 3'
<i>sup45-102</i>	Y53	TTA ACA GAT GAA TAA GGT ACT GCC TCG
<i>sup45-101, 20</i>	E266	AAC CAG GCT ATC TAA CTT TCT GCC GAA
<i>sup45-104</i>	L283	CAA GAA AAG AAA TAA TTG GAG GCA TAT
<i>sup45-105</i>	E385	GGT GCT ACC TTG TAA TTC ATC ACA GAC
<i>sup45-13</i>	E266	GCT ACC GGC CAA TAA ATG GAC GTT GTC
<i>sup35-240</i>	Q56	GGT TCT GGG TAC TAA CAA GGT GGC CAA
<i>sup35-218</i>	E181	GCT GAA ACC AAA TAA CCA ACT AAA GAG
<i>sup35-13, 215</i>	K102	CGT GGA AAT TAC TAA AAC TTC AAC TAC
<i>sup35-231</i>	Q631	CAA GAT TAC CCT TAA TTA GGT AGA TTC
<i>sup35-21</i>	Q422	GAA CGT TAC GAC TAA TGT GTG AGT AAT
<i>sup35-74</i>	Q129	TTG AAC GAC TTT TAA AAG CAA CAA AAG
<i>sup35-201</i>	K516	GGT CAT ATC AAA TAG GGT CAA TCC ACC
<i>sup35-260</i>	Q242	TTG ATC AAG GAA TAG GAA GAA GAA GTG
<i>sup35-244</i>	E197	CCA GTT AAA AAG TAG GAG AAA CCA GTC
<i>sup35-203</i>	Q72	GGT TAC CAG CAA TAG TAT AAT CCT CAA
<i>sup45-107</i>	L317	GCA GTC GAA AAA TGA ATT GTT TTC GAA

* CAA codons are underlined, adenines preceding nonsense mutations and promoting the translation of stop codons in yeast cells (Tork et al., 2004) are highlighted in yellow. Nucleotides at position +4, contributing to and inhibiting the translation of the stop codon (Bonetti et al., 1995) are highlighted in blue and green, respectively.

firms numerous data on the role of this domain in translation termination.

The suppressor phenotype of isolated missense mutations cannot be explained by a reduced amount of eRF3 or the inability of mutant eRF3 to interact with eRF1. These mutations may affect the interaction of eRF3 with other proteins or the ribosome.

All missense mutations obtained result in amino acid substitutions that are conservative between *S. cerevisiae* and *S. pombe* eRF3. The exception was one of the substitutions in the case of the double *sup35-233* mutation (G365V; E559K): N residue was substituted for E residue in the *S. pombe* eRF3 in the homologous position, making it possible to arrange the corresponding amino acid substitutions in the tertiary structure of the C-terminal domain of *S. pombe* eRF3 (Kong et al., 2004).

The *sup35-228* mutation results in arginine being replaced with lysine at position 372. While being very similar chemically, these two amino acids differ in their methylation capacity. A number of studies have shown a simple replacement of arginine with methylated lysine to result in a defective protein, with developmental disorders of transgenic tobacco plants as a result of a mutation in the calmodulin gene (Roberts et al., 1992) and a sharp decrease in RNase activity in the case of human mutant angiogenin (Shapiro et al., 1989).

It should be noted that about half of the *sup35* mutations reported in the works of different authors are located either in the *SUP35* regions encoding the GTPase domains of eRF3 or in the immediate vicinity of these sites (Fig. 1C, Table 1). Interestingly, the phenotypic manifestation of mutations located in the C-terminal domain of eRF3 may be influenced by the presence of the N-terminal domain of eRF3 (Volkov et al., 2007). The same mutations obtained on the background of a full-sized or truncated *SUP35* had a different phenotypic manifestation (Volkov et al., 2007). The interaction of this site with the mutant C-terminal domain may reduce the GTPase activity of eRF3, resulting in the suppression efficiency increase.

The nature of the suppression was investigated in details in two mutants in the GTPase domain of yeast eRF3 (Salas-Marco and Bedwell, 2004), by studying the mutants of eRF3 with impaired hydrolysis of GTP. If the functions of eRF3 coincide with those of RF3, the eRF3 mutations leading to the nucleotide exchange rate decrease should also slow down the release of eRF1 from the termination complex. This, in turn, should lead to a decrease in the pool of eRF1 available for subsequent termination events. At the same time, mutations that damage the GTP hydrolysis rate should be defective in the dissociation of eRF3 from the ribosomes. In this case, the pool of free eRF3 should decrease, thus decreasing eRF1 recycling. In the work concerned, six *sup35* mutations (V269G, H348L, H348Q, K407E, D409W, and

R419G) were obtained using site-specific mutagenesis. Only two of the six mutant proteins (eRF3-H348Q and eRF3-R419G) proved to be able to maintain the viability of yeast cells, although their presence resulted in a decrease in the growth rate. H348Q and R419G mutations, decreasing the rate of GTP hydrolysis, resulted in significant impairment of translation termination only on the UGAN stop codons (the stop codon translation level, being a significant one, increased by 3.7–16.8 times depending on the stop codon). At the same time, most of UAGN or UAAN codons maintained their termination codon properties in the presence of eRF3-H348Q or eRF3-R419G mutant proteins. The only exceptions were the tetranucleotides UAGC and UAAC, with the translation of the stop codon being 3.2–4.4 times higher than that of the wild-type eRF3 (Salas-Marco and Bedwell, 2004). Thus, the detection of eRF3 mutations that disrupt both GTPase activity and the recognition of specific stop signals provides evidence for eRF3 functions not to be limited to the simple recycling of eRF1. N406I or D409N substitutions in GTP-binding motif NKXD reduced affinity for GTP and eRF1–eRF3 association was markedly impaired (Kobayashi et al., 2004).

A potential phosphorylation site has been identified in the C-terminal Sup35 domain. It was found that the threonine residue at position 341 can be phosphorylated by protein kinase A *in vitro*. Provided this residue is replaced by asparagine (constitutive phosphorylation) or alanine (lack of phosphorylation), the translation termination accuracy is reduced by 4 and 2 times compared to wild protein type, respectively, with a disruption of the interaction of Sup35 and Sup45 and a decrease in GTPase activity. It is noteworthy that unlike T341A, T341D reduced cell viability whereas, in the absence of the N-domain, both mutant proteins were able to compensate for the disruption of the wild-type allele (Fabret et al., 2008). In subsequent work, it was shown that the T341D replacement causes synthetic lethality in combination with a prion [*PSI*⁺], and the T341A mutation results in a weakening of [*PSI*⁺]. Thus, the authors showed the influence of the C-domain on the [*PSI*⁺] properties and its role in the formation of fibrils (Kabani et al., 2011).

3.2.3. Other mutations in the *SUP35* gene

The *sup35-217* mutation results in the duplication of six amino acid residues (VNKMDL) (Table 1), forming one of the GTP-binding motifs (N₄₀₆K₄₀₇D₄₀₉) (Kong et al., 2004). The mutation *sup35-50* (T412P) is localized in the same region. It is possible that these mutations disrupt the GTPase activity of eRF3, resulting in suppression. Mutations in the GTP-binding motif (N153KMD156) of the yeast translation elongation factor eEF1A are known to cause suppression of all three stop codons (Carr-Schmid et al., 1999).

In the case of *sup35-222* mutation which is characterized by omnipotent nonsense suppression and reduced levels of Sup35 and Sup45 proteins, we could not detect any change in the coding region of *SUP35* (Chabelskaya et al., 2004). Using whole-genome sequencing of the *sup35-222* mutant strain one single-nucleotide variation was found 183 bp upstream of the *SUP35* coding sequence (Table 1). It was suggested that this substitution destroys potential Abf1-binding site in the *SUP35* promoter (Matveenkov et al., 2019).

Sequence polymorphism of the *SUP35* gene of the Peterhof genetic line (PGL) and the sequence presented in GenBank (S288C) was detected. All polymorphic sites were located in the non-conserved NM-domain and did not have their own phenotypic manifestation (Volkov et al., 2000).

4. Termination factor eRF1

4.1. eRF1 STRUCTURE

The structure and sequence of eRF1 are highly conservative (Frolova et al., 1994) although some organisms have several eRF1 paralogs that may vary in specificity of stop codon recognition (Liang et al., 2001; Chapman and Brown, 2004; Kim et al., 2005; Atkinson et al., 2008; Kim et al., 2008). The eRF1 protein consists of three domains: N, M, and C (Fig. 2, 3A).

The TASNIKS and YxCxxxF motifs, which are likely to play the role of specific “protein anticodons”, are required for the N-terminal domain to recognize a stop codon (Song et al., 2000; Frolova et al., 2002; Salas-Marco and Bedwell, 2004; 2006; Fan-Minogue et al., 2008; Cheng et al., 2009). The same domain is required for binding to the ribosome (Chavatte et al., 2001). The central M-domain with a conservative GGQ motif provides peptidyl-tRNA hydrolysis (Frolova et al., 1999; Seit-Nebi et al., 2001). The C-terminal region of eRF1 containing the amino acid sequence GFGGIG (G/A) XLRY is responsible for the eRF1–eRF3 binding (Eurwilaichitr et al., 1999; Merkulova et al., 1999).

X-ray analysis of human eRF1 and *S. pombe* (Song et al., 2000; Cheng et al., 2009) has shown the eRF1 molecule to resemble the tRNA molecule in three-dimensional structure, confirming the hypothesis of molecular mimicry proposed earlier for prokaryotic release factors (Ito et al., 1996).

4.2. SUPPRESSOR MUTATIONS IN THE *SUP45* GENE

A specific feature of class-1 release factors is their ability to recognize stop codons. Disruption of this function can also appear in the form of a suppressor effect, suggesting that the study of suppressor mutations can provide information on the mechanism of recognition of stop codons by class-1 termination factors. The molecular mimicry

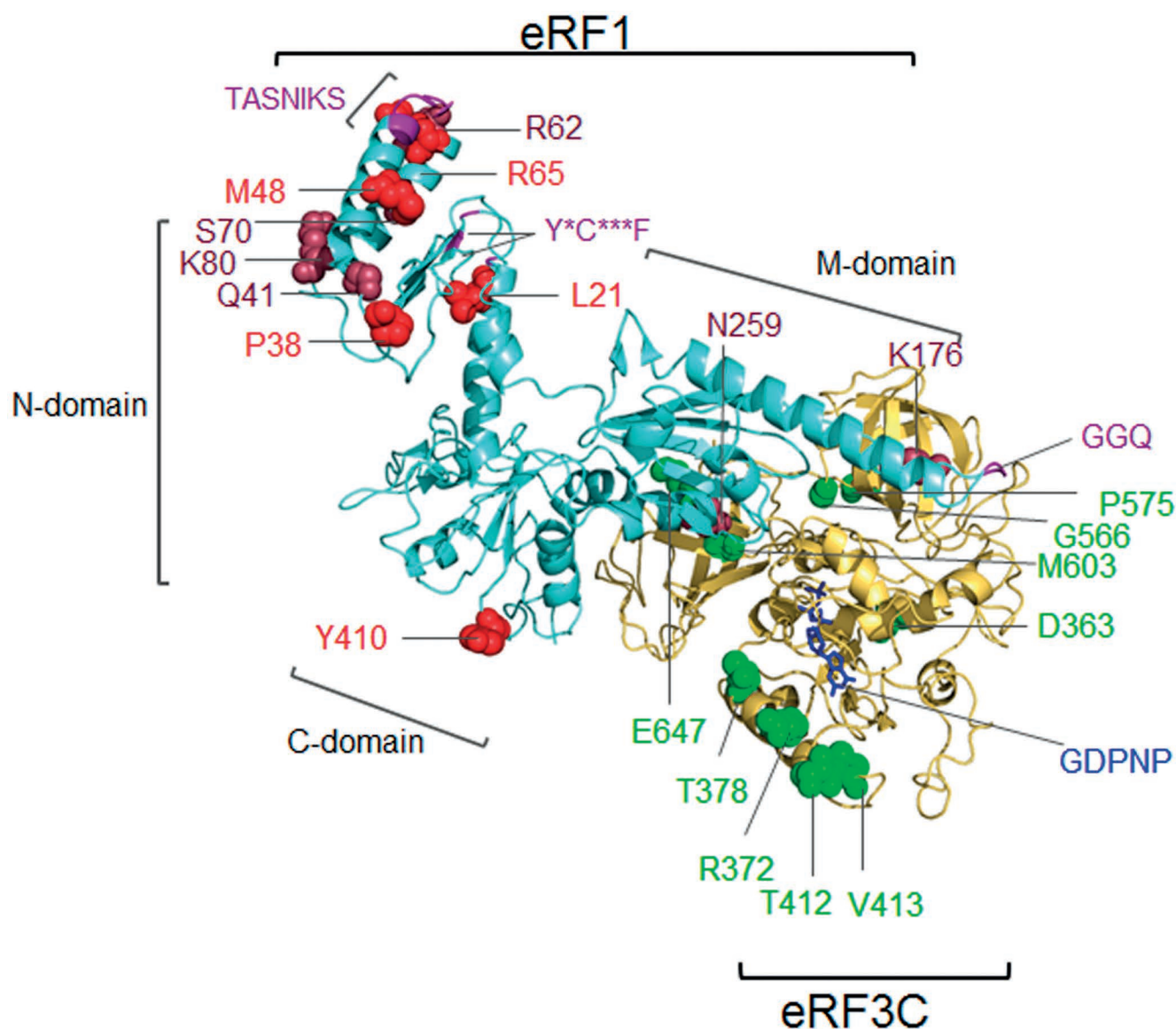


Fig. 2. Suppressor missense mutations in *SUP45* and *SUP35* genes studied in our laboratory.

All the mutations were denoted on 3D model of eRF1-eRF3C-GDPNP protein complex of *S. cerevisiae* (Preis et al., 2014). eRF1 (containing N, M and C domains) and eRF3C (corresponding only C-terminal part of eRF3) are shown in aquamarine and yellow orange colors, respectively. Mutations in *SUP45* gene that were also obtained in different works (see Table 4) are shown in red spheres (“hot spot”); while mutations obtained only once are shown in raspberry spheres. All single mutations *sup35* obtained in our laboratory are shown in green spheres (see Table 1). The NIKS motif, the YxCxxxF motif and the GGQ motif of eRF1 are shown in purple ribbons. Nonhydrolyzable GTP analog guanylyl imidodiphosphate (GDPNP) is shown in blue sticks. The figure is generated by PyMOL (<https://www.pymol.org/pymol>).

hypothesis implies that class-1 release factors mimicking the structure of tRNA include “peptide anticodon” responsible for recognition of the stop codon and tRNA corresponding to the anticodon (Ito et al., 1996).

The selection of mutations in the *SUP45* gene was performed by simultaneous reversion of nonsense TAA mutations located in two different genes (*HIS7* and *LYS9*) (Moskalenko et al., 2003). To select the mutations, two different strains were used. The selection system specificity was associated with the search for such mutations in the *SUP45* gene that would disrupt the interaction of eRF1 with only one of the three stop codons (in

this case, the UAA). Sequencing the resulting mutations would allow identification of the eRF1 region responsible for recognizing nonsense codons.

4.2.1. Nonsense mutations in the *SUP45* gene

We have characterized six *sup45* nonsense mutations, with two of them located in the region of the *SUP45* gene encoding the NM-domains of eRF1 (Table 4, Fig. 3B). Three *sup45-n* mutations are located in the last third of the *SUP45* gene, encoding the C-terminal domain of eRF1. Figure 3B shows that most of the nonsense

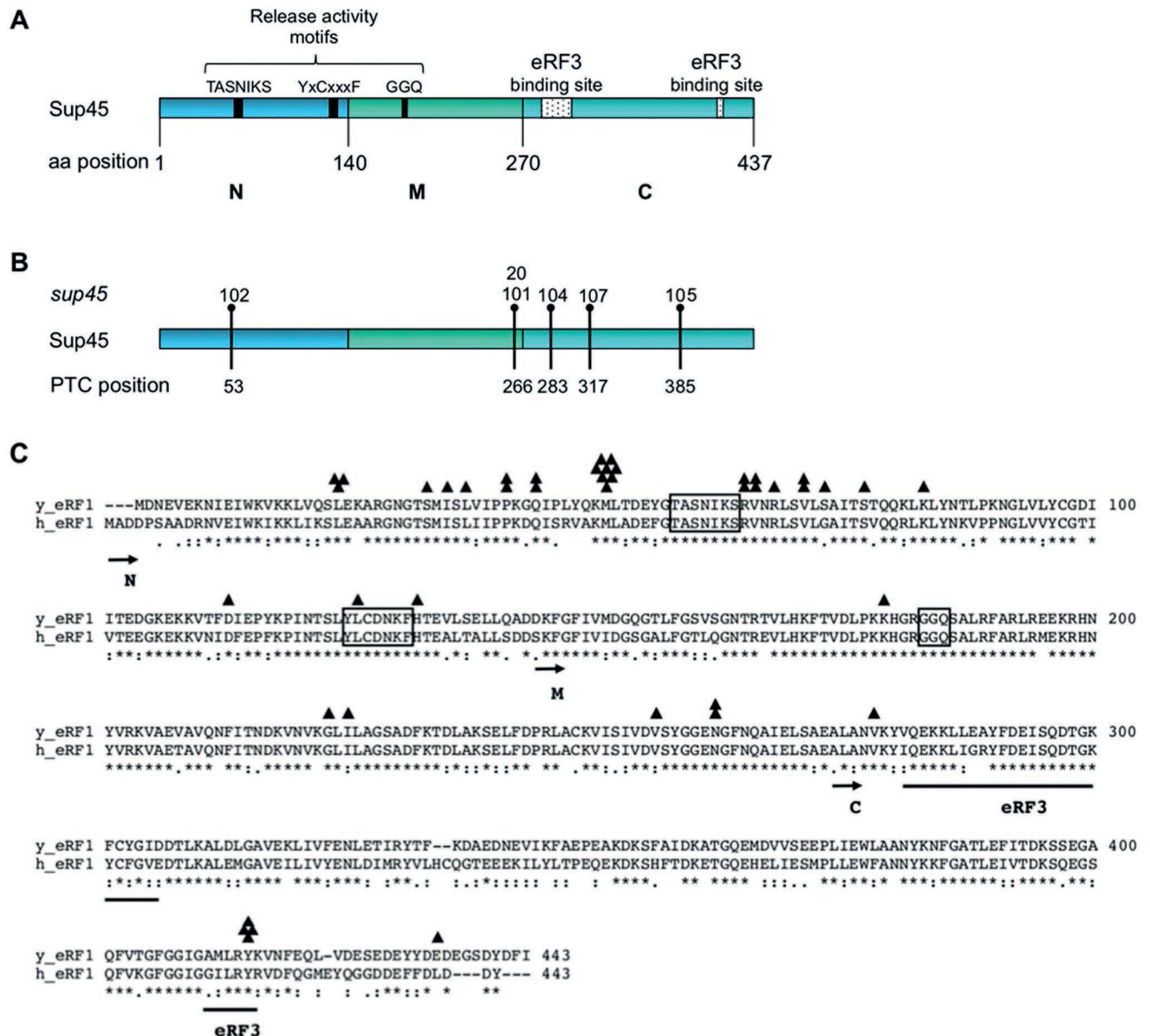


Fig. 3. The mutations obtained in the *SUP45* gene.

A. Sup45p consists of three domains: N, M and C (Kisselev et al., 2003). N-domain includes two conservative release activity motives: TASNIKS and YxCxxF, which are required for stop codon recognition. M-domain contains conservative eRF3 binding site performing peptidyl-tRNA hydrolysis. C-domain contains two eRF3 binding sites, the last one stimulates GTPase activity of eRF3 (Eurwilaichitr et al., 1999; Merkulova et al., 1999). **B.** Nonsense mutations in *SUP45* gene obtained in our laboratory (Moskalenko et al., 2003). Numbers above Sup45 protein scheme correspond to numbers of the *sup45* mutations. PTC (premature termination codon) positions match to the aa length of truncated proteins formed in the case of translation termination on the *sup45* mutations. **C.** Suppressor missense mutations in *SUP45* gene obtained in different laboratories (Breining and Piepersberg, 1986; Mironova et al. 1995; Stansfield et al. 1996; Bertram et al. 2000; Bradley et al 2003; Moskalenko et al., 2004; Valouev et al. 2004). Alignment of *S. cerevisiae* and *H. sapiens* eRF1s is shown. The triangle signs correspond single missense mutations (stacks of them correspond to hotspot mutation sites). Arrows below N, M or C mean the start of corresponding domain of Sup45. TASNIKS, YxCxxF and GGQ-motives are framed; eRF3 binding sites are underlined.

sup45 mutations, isolated by various authors, are located precisely in the last third of the *SUP45* gene.

4.2.2. Missense mutations in *SUP45* gene

All the *sup45* missense mutations, isolated on strain 1B-D1606, are localized in the N-terminal eRF1 domain (Fig. 3C), confirming numerous data on the role of this

domain in stop codon recognition. Five out of eleven isolated *sup45-m* missense mutations result in methionine being substituted by isoleucine in the 48 position. That this region of the eRF1 protein is a "hot spot" confirms the fact that similar mutations were obtained by other authors by using different approaches for isolating mutations in the *SUP45* gene. It should be noted that in our work, the unipotent phenotype with growth on

Table 4. Mutations in the *SUP45* gene, described in different works.**X means substitution of amino acid to stop codon; † indicate lethality of the mutation; * only single mutations are listed**

Method of selection	Type of mutation	Nucleotide changes	Amino acid changes	Reference
Suppression of <i>ade1–14 (UGA)</i> and sensitivity to paromomycin (strain 7B-D244)	Missense	T101C	L34S	Breining and Piepersberg, 1986
Suppression of <i>ade1-14(UGA)</i> and <i>his7-1(UAA)</i> (strain P3990)	Missense	C193T	R65C	Mironova et al., 1995
EMS mutagenesis (strain BSC483/la)	Nonsense	C136T; C175T; C1078T; A1231T	Q46X; Q359X; Q360X; K411X	Stansfield et al., 1996
Allosuppressor of <i>SUQ5</i> (strain MT552)	Missense	T665G	I222S	Stansfield et al., 1995b
Suppression of <i>ade1–14 (UGA)</i> (strain IS31D7b/ 1c) or <i>ade2-1(UAA)</i> (strain TGB7a/5b)	Missense	A94T; C113T; G144A; T203C; A219T; A320G; N367G; A386G; G1282C	I32F; P38L; M48I; V68I; V68A; S74F; D110G; L123V; H129R; E428Q	Bertram et al., 2000
	Nonsense	C1243T	Q415X	
Suppression of <i>ade1–14 (UGA)</i> (strain 74-D694)	Missense	G144T; G187T; T188A; G659A; G757T; T824A; A1229C	M48I; V63F; V63D; G220D; V253F; V275D; Y410S	Bradley et al., 2003
	Nonsense	G366A; G1115A; G1243T	E366X; W372X; E414X	
Suppression of <i>lys9-A21(UAA)</i> and <i>his7-1(UAA)</i> (strain 33G-D373)	Nonsense	G796T	E266X	Our unpublished data
	Missense	G528T; C777A (two times); A1229G (three times)	K176N; N259K (two times); Y410C (three times)	
Suppression of <i>ade1–14 (UGA)</i> and <i>his7-1(UAA)</i> (strain 2V-P3982)	Missense	T62C (two times); C113T; A122C (two times); G144T; G240C	L21S (two times); P38L; Q41P (two times); M48I; K80N	Moskalenko et al., 2004
Suppression of <i>lys9-A21(UAA)</i> and <i>his7-1(UAA)</i> (strain 1B-D1606)	Missense	T62C; G144T (five times); G185C (two times); C209T	L21S; M48I (five times); R62T (two times); S70F	
	Nonsense	T159A; G796T; T848A; T950G; G1153T	Y53X; E266X; L283X; L317X; E385X	Moskalenko et al., 2003
Spontaneous mutagenesis (strain 33G-D373)	Missense	T67C; G228C/C229T	S30P; Q76H/Q77X	Valouev et al., 2004
Spontaneous cryptic mutations (strains 4V-P4482 and 10-2V-P3982)	Missense	G1199T C224T	G400V T75I	Aksenova et al., 2006
Site directed mutagenesis	Missense	T1261G; T1294G; T1261G/T1294G; TC1261GA; TC1294GA; TC1261GA/TC1294GA	S421A; S432A; S421A/S432A; S421D; S432D; S421D/S432D	Kallmeyer et al., 2006
Site directed mutagenesis	Nonsense	C951T	L317X	Zhouravleva et al., 2007
Site directed mutagenesis	Missense	T544G; T544G/G(A)546C(T)	Q182E; Q182N	Studte et al., 2008
Site directed mutagenesis	Missense	A1229C	Y410S	Akhmaloka et al., 2008
Site directed mutagenesis	Nonsense	C228T; C726T	Q76X, R242X	Kiktev et al., 2009
	Missense	TA157GG; A1154G; A1155T	Y53Q; E385Q; E385Y	
PCR mutagenesis	Missense	A79T; G151A; T174A; C226A; A227G; A257G; G310A; T325C; A641G; A1079T; A1079G	N27Y; D51N; N58K; Q76K; Q76R; K87E; E104K; F109L; D214G; E360V; E360G	*Hatin et al., 2009
PCR mutagenesis	Missense	CT148GC; A158N; G163A†; A165G; AA174GT; AA180GT†; AG183GT; AG186GT; T322A; N523F; AA525GT†; AA528GT†; G541T†; G544T†; A886G; A915G; A1071G; A1164G; T1204A; A1231T	L49A; E52A; G54D†; T55A; N58A; K60A †; S61A; R62A; V107D; P174Q; K175A †; K176A †; G180A †; G181A †; T295A; T305A; T357A; T388A; F401Y; Y410F	Merritt et al., 2010

selective media characterized both the *sup45-113* mutation (M48I) and the *sup45-115* mutation (S70F). Mutation M48I also was described as unipotent by Bertram et al. (2000). At the same time, this mutation is reported as omnipotent on a different genotypic background (Moskalenko et al., 2004).

We compared the distribution of the amino acid substitutions in the eRF1 protein described in the literature that were caused by missense mutations. As can be seen from Figure 3C and Table 4, the N-domain positions, in which missense mutations in the *SUP45* gene are found with different selection methods, are the same for many authors.

We have found two mutations (S70F and R62T) in N-domain of Sup45 that has not been described previously in the literature. A substitution identified by sequencing the first mutant *SUP45* allele (L34S) (Breining and Piepersberg, 1986) was in the same region. Also, one more mutant (*sup45-3* containing R65C) was described in our laboratory earlier (Mironova et al., 1995). It makes it possible to conclude that there are only a few positions in the eRF1 protein where substitutions are not lethal and lead to nonsense suppression.

The remaining six mutations selected in our work on strain 33G-D373 result in amino acid substitutions in three sites, with one being in the central part of the yeast eRF1 (position 176), and two at the C-end of the molecule (position 259 and 410). Position 176 is close to one of the putative eRF1 interaction sites with eRF3 in *S. pombe* (amino acid residues 187–247 (Ito et al., 1998)). According to our data, a mutation in this position disrupts the interaction of eRF1 and eRF3 in the two-hybrid system, possibly leading to the effect of nonsense suppression. Since the C-terminal amino acid residues of eRF1 are actively involved in the interaction with eRF3, the effect of mutations at positions 259 and 410 may also be associated with a disruption of this interaction. It was shown that substitution Y410S decreased binding affinity of eRF1 to eRF3 up to 20% of the wild type (Akhmaloka et al., 2008).

Thus, the distribution pattern of the mutations studied allows one to conclude that most of the missense mutations in the *SUP45* gene, selected by the nonsense suppression effect, result in amino acid substitutions in the N-terminal part of the eRF1 molecule, limited by 21–80 positions. Mutations selected by the suppression effect of a nonsense mutation of the same type (TAA) can result in aa substitutions in different parts of the molecule, or they represent nonsense mutations in the *SUP45* gene (Moskalenko et al., 2003). However, even in this variant of selection, most missense mutations affect the same positions in the eRF1 protein as mutations selected by the “classical” method. It is also significant that none of the missense mutations studied reduced the amount of eRF1 protein.

It is interesting to compare our data with the data obtained in the Stansfield laboratory (Bertram et al., 2000), where the mutants for the *SUP45* gene were selected by using a library of mutagenized plasmids carrying the *SUP45* gene. The goal of this work was to identify the mutant *SUP45* alleles exhibiting a “unipotent” suppressor effect that suppresses only one type of nonsense mutations. However, although the mutations obtained had different codon specificity at the phenotypic level, it was found that in their presence, all three stop codons were actually read, albeit with different efficiencies (Bertram et al., 2000). Most of the mutations obtained in this work also affect the N-terminal part of the protein (positions 32, 38, 48, 68, 74). At the same time, some of them affected the same positions in the protein as the mutations studied by us (positions 38 and 48). In addition, three amino acid substitutions were located somewhat to the right of the site marked in our work (positions 110, 123, 129), with one more substitution located at the C-end of the molecule (position 428).

This work focused mainly on mutations affecting the N-terminal part of the molecule. In addition to our data suggesting that the mutations in question did not affect the interaction with the eRF3 protein, it was shown that these mutants were not influenced by the binding to the ribosome. Thus, the most likely function impaired by amino acid substitutions in the N-terminal part of the eRF1 protein is the interaction of eRF1 with stop codons.

The involvement of the N-terminal domain in recognition of a stop codon was shown *in vivo* (Bertram et al., 2000) since mutations in this region resulted in a change in the specificity of stop codon recognition by the eRF1 protein. Genetic screening of mutants characterized by a unipotent suppressor phenotype (that is, leading to the suppression of only one stop codon) revealed a series of missense mutations (Bertram et al., 2000) (Fig. 3C, Table 4). Most of them were located in the gap between the two α -helices and the opposite β -layer of the N-domain eRF1. It is hypothesized that it is these structures that form the surface of eRF1 involved in the interaction with mRNA. With all isolated mutants revealing a weak omnipotent suppressor phenotype, a hypothesis was put forward that stop codon decoding in eukaryotes is an integral process—that is, the recognition of a single nonsense codon cannot be completely separated from the recognition of other termination codons. This hypothesis suggests that stop codon decoding in eukaryotes is more complex and therefore different from sense codon decoding using tRNA (Bertram et al., 2000).

Additional data concerning the molecular mechanisms involved in the discrimination of stop codons were obtained in experiments using proteins of the eRF1 family from organisms using the UAG codon or the UAA

and UAG codons as sense codons (see the review by Lozupone et al., 2001). It is evident that eRF1 should no longer recognize UAG (or UAA and UAG) in these organisms as translation termination signals. In this regard, it was suggested that amino acid residues that determine the specificity of eRF1 to a specific stop codon could be identified by comparing the eRF1 sequences in organisms with non-standard genetic code with organisms that have a universal genetic code (Karamyshev et al., 1999). The comparison of sequences did allow a number of hypotheses explaining the decoding process to be formulated (Inagaki and Doolittle, 2001; Lozupone et al., 2001; Muramatsu et al., 2001; Inagaki et al., 2002). Unfortunately, these models were not confirmed by experiment, with most of the experiments making it possible to establish that the recognition of a stop codon is not a linear sequence of amino acid residues eRF1, but more complex structural motif (s) (Ito et al., 2002; Seit-Nebi et al., 2002; Chavatte et al., 2003; Salas-Marco et al., 2006).

A large collection of point mutations in yeast *SUP45* was obtained and characterized (Hatin et al., 2009). Among them were mutations that significantly increased translation termination efficiency, some of which are listed in Table 4. However, most of the mutants contained two or three aa changes (Hatin et al., 2009). From twenty new *sup45* mutants six were inviable (Table 4) (Merritt et al., 2010). A large number of interesting conclusions came from this work, including strain-specific differences in the stop codon read-through, and between *in vivo* and *in vitro* experiments. Extensive characterization of *sup45* mutants from different collections as well as new ones allowed authors to show that various phenotypes associated with these mutants are independent of defects in translation termination (Merritt et al., 2010).

Using site-directed mutagenesis, Sup45 phosphorylation sites were identified (Kallmeyer et al., 2006), and the effect of Sup45 methylation on translation termination accuracy was shown (Studte et al., 2008).

5. Appearance of nonsense mutations in *SUP45* and *SUP35* genes

In our work, nonsense mutations in the essential *SUP45* and *SUP35* genes were shown for the first time not to cause lethality in the absence of suppressor tRNA. Currently, several processes are known that can overcome the negative effect associated with the presence of nonsense codons. In particular, the presence of mutant (and in some cases non-mutant) tRNA in the cell results in the suppression of stop codons (see the review by Beier and Grimm, 2001). The so-called “weak” termination context and/or the RNA secondary structure specificity can also result in stop codon translation (see

review Bertram et al., 2001). In our work it was shown that viability of *sup45* nonsense mutants is supported by diverse mechanisms that control the final amount of functional Sup45 in cells (Kiktev et al., 2009).

Many laboratory strains, as well as natural populations of *E. coli*, contain suppressor tRNA^{UAG} (Tate et al., 1996). The mutation rate in at least some tRNA genes is known to be significantly increased (Ito-Harashima et al., 2002). It is highly unlikely that the initial strains 33G-D373 and 1B-D1606 used in our work contain mutant suppressor tRNA. Firstly, such suppressors are usually codon-specific, and their presence would result in the appearance of nonsense *sup45* or *sup35* mutations of only one type (for example, TAA). In our work, mutations of different types were isolated: (TAA) and (TGA), in the case of *sup45-n*, and (TAA) and (TAG) for *sup35-n*. Secondly, all the *sup45-n* and *sup35-n* mutations described in our work are recessive, while mutations in the tRNA genes are usually dominant. All *sup35-n* transformants selected during plasmid shuffle experiments and containing nonsense *sup35* mutations on the plasmid retained their recessive suppressor phenotype, while in the case of additional mutations this effect should be dominant. Moreover, it is known that suppressor mutations in tRNA genes are generally incompatible with *sup35* and *sup45* mutations (Song and Liebman, 1985). Thirdly, the parent strains, as well as the strains used to assess the viability of the *sup45-n* and *sup35-n* mutations, did not possess the tested *in vitro* suppressor activity. In strains 1A-D1628 and 16A-D1608 used in plasmid substitution experiments, no suppression of *ade1-14* (TGA), *his7-1* (TAA), *trp1-289* (TAG) and *lys9-A21* (TAA) mutations was found. In addition, the sequenced genome of strain S288C, the isogenic derivatives of which were used in plasmid substitution experiments, does not contain mutations in the tRNA genes (Percudani et al., 1997; Drozdova et al., 2016).

Non-lethal nonsense mutations in the *SUP35* and *SUP45* genes were described by other authors (Zhou et al., 1999; Bradley et al., 2003), but in those cases, it remained unknown whether the parent strain possessed any suppressor activity. The nonsense mutations in the *SUP45* (*sup45-0*) gene were also isolated in the presence of the *SUQ5* mutation, resulting in the appearance of a suppressor tRNA^{Ser} in the cell; but these mutations were lethal in the absence of the *SUQ5* mutation (Stansfield et al., 1996). We have estimated the content of “potential” stop codons (codons, single mutations in which can lead to the appearance of stop codons) in the *SUP45* gene (Table 2). In the case of codons, single mutations in which can lead to the appearance of two different stop codons (for example, replacing a TTA codon in the second position will result in a TAA codon or TGA), the corresponding frequencies were equally divided between the two classes. The data given in Table 2 indicate that po-

tential stop codons are fairly evenly distributed across the *SUP45* gene sequence. In the regions encoding the N, M and C-domains, such codons account for 39.1 %, 30.8 % and 38.3 % of the total number of codons, respectively.

Thus, the uneven distribution of nonsense mutations in the *SUP45* gene cannot be accounted for by the increased codon content, a single substitution in which can result in the appearance of a stop codon. It is possible that the preferred localization of nonsense mutations in the region encoding the C-domain is related to the nucleotide context features. The absence of mutations resulting in the appearance of the stop codon UAG among the *sup45-n* mutations can be explained by the specificity of selection carried out by the simultaneous reversion of two other mutations. At the same time, in the screening of Ade⁺ revertants (a strain containing the *ade1-14* mutation (TGA) was used), two *sup45-n* TAG mutations and one TAA were isolated (Bradley et al., 2003). The fact that only one of the five *sup45-n* mutations isolated (Moskalenko et al., 2003) caused the appearance of the TGA codon can be explained by the reduced content of potential UGA codons in the *SUP45* gene sequence. Indeed, the *SUP45* gene sequence contains 14 % of such codons as compared with 33 % and 53 % for potential codons UAG and UAA, respectively (the number of potential stop codons is taken as 100 %) (Table 2).

The termination efficiency is known to depend on the local context surrounding the stop codon (see reviews by Tate and Mannering, 1996; Kisselev and Buckingham, 2000; Bertram et al., 2001). Although the local context in yeast and bacteria is different, there is a fairly strong correlation between the frequency of a particular nucleotide being present at position +4 in the natural termination sites and the termination efficiency at the corresponding tetranucleotide stop codon (Bonetti et al., 1995; Poole et al., 1995). According to these data, the relative level of the full-length eRF1 protein correlated with the strength of the translation termination signal: in the case of the strongest (UAAG) and weakest (UAAC) termination signals, the number of eRF1 was 8 % and 32 % of the wild-type level, respectively (Moskalenko et al., 2003).

In the case of the UGA codon, the translation termination efficiency decreases in the order G > U > A > C depending on the position (+4) while for the UAG codon, this order is different (A > U > C > G) (Bonetti et al., 1995). Indeed, the *sup45-107* mutation (TGAA) is located in a weak terminating context. At the same time, the replacement of the TGA codon by TAG in the mutant *sup45-107* allele led to a change in the local context (TGAA → TAGA) and lethality (Moskalenko, 2003). Thus, the *sup45-n* mutations can be supposed to be located in a “weak” terminating context.

It should be noted that all *sup45-0* mutations that caused the UAA codon to appear in the presence of

SUQ5 (Stansfield et al., 1996) were located in a “strong” context since purine was in the +4 position of these mutations. However, since one of our viable *sup45-n* mutants (*sup45-13*) contained an allele identical to *sup45-18* (Stansfield et al., 1996), the inviability of the *sup45-0* mutants in the absence of *SUQ5* cannot be explained by the influence of the context. A mathematical model predicting the Sup45 translational readthrough negative feedback loop control was created; this model anticipates that manifestation of *sup45-n* mutations will depend on nucleotide context, as well as on the level of tRNA suppression and amount of *SUP45* mRNA (Betney et al., 2012).

We have not found an apparent correlation between the location of the nonsense mutation in the *SUP35* gene coding sequence and the amount of the full-length eRF3 protein. It is known that not only the nucleotide at position +4, but also the other nucleotides surrounding the stop codon, affect the efficiency of translation termination (Mottagui-Tabar et al., 1998; Cassan and Rousset, 2001; Namy et al., 2001; 2002; Liu, 2005). It is assumed that this effect is due to the interaction of these mRNA sites with various components of the translation apparatus (see review by Tate et al., 1996; Bertram et al., 2001). We have compared the nucleotide environment of stop codons for the nonsense *sup45* and *sup35* mutations that were isolated in our works (Table 3).

Analysis of the nucleotide context revealed that among 16 *sup45-n* and *sup35-n* mutations, 8 contain adenine at position -1, 12 contain adenine at position -2, and 8 contain adenine in both positions. The presence of two adenines immediately before the stop codon was shown to facilitate translation of the stop codon in yeast cells (Tork et al., 2004). Some of the mutations are surrounded by codons CAA, which are known to increase the level of suppression (Kopczynski et al., 1992; Bonetti et al., 1995). It is possible for all the *sup45-n* and *sup35-n* mutations to be located in a weak terminating context.

6. Suppression due to prion [*PSI*⁺]

Sup35 protein aggregation in a yeast cell leads to the appearance of a prion [*PSI*⁺]. In order to be considered a prion, a genetic determinant must satisfy a number of genetic criteria (Wickner et al., 1995). One of them is the similarity of phenotypes in mutants by the structural gene of the prion and prion-associated phenotypes. The main phenotypic manifestation of the [*PSI*⁺] factor is a decreased translation accuracy (see review Cox et al., 1988), which is similar to the mutation effect in the *SUP35* gene, the structural gene of [*PSI*⁺]. Different strains of [*PSI*⁺] exhibit different suppression efficiencies (Derkatch et al., 1996) which can vary from 0.2 to 35 % (Bidou et al., 2000; True et al., 2000; Uptain et al., 2001; Kiktev et al., 2009). Early works argued that the [*PSI*⁺]

factor does not affect the growth rate of yeast strains (see the review by Cox et al., 1988). Studies conducted on seven yeast strains using 150 different culture conditions showed that in 50% of cases $[PSI^+]$ did not really affect growth, while the other cases demonstrated both growth inhibition and stimulation in the presence of $[PSI^+]$ (True et al., 2000). In some cases, the $[PSI^+]$ factor led to a change in the colony morphology (True et al., 2000) and cell sensitivity to paromomycin at 20°C (Cosson et al., 2002). A systematic comparison of the phenotype of $[PSI^+]$ strains with isogenic $[psi^-]$ strains, as well as isogenic strains carrying the *sup35* (C653R) mutation, in 70% of cases, revealed a coincidence of $[PSI^+]$ -induced phenotypes with phenotypes caused by the *SUP35* mutation. At the same time, in 30% of cases, such a correlation was not observed (True et al., 2004). This fact allowed the authors to suggest that the presence of $[PSI^+]$ factor in yeast cells can provide them with selective advantages due to the expression of pseudogenes or 3' untranslated RNA under normal conditions (see the review by Shorter and Lindquist, 2005). Despite the fact that this hypothesis remains to be proved and provokes active criticism from a number of researchers (see, for example, Nakayashiki et al., 2005), it is obvious that in some cases the phenotype caused by the presence of the factor $[PSI^+]$ may differ from the phenotype caused by the *SUP35* mutations. A possible explanation for such differences may be that in the cited work (True et al., 2004), the phenotypes caused by the presence of $[PSI^+]$ were compared with phenotypes of only one *sup35* mutation. Various *sup35* mutations are known to reveal various manifestations, including thermosensitivity or its absence, different suppression efficacy, sensitivity or resistance to benomyl, paromomycin, etc. The reasons for these differences are not currently known. At the same time, the $[PSI^+]$ factor, originally described as the allo-suppressor *SUQ5* (Cox et al., 1988), does not on its own suppress all nonsense mutations. Hence, early studies reported $[PSI^+]$ not to suppress TAG mutations and most TAA mutations (Liebman and Sherman, 1979). It should be noted that the quantitative assessment of the suppression effectiveness that showed $[PSI^+]$ to suppress all three stop codons was carried out on a strain containing the *SUQ5* mutation (Firoozan et al., 1991). The mutant tRNA^{Ser} resulting from the *SUQ5* mutation is known not only to suppress the UAA codon but, in the presence of the *sal4-2* mutation, to have an allosuppressive effect on the UAG codon (Stansfield et al., 1995b). The $[PSI^+]$ factor has been reported to suppress mutations in *cyc1-72*, *trp5-48*, *cyc1-179* (TAA) (Liebman and Sherman, 1979), *met8-1*, *lys2-A12* (TAG) (Ono et al., 1986; Chernoff et al., 1995), *lys2-101* (TGA) (Ono et al., 1986), *lys2-87*, *ade1-14*, *thr4-B15* (TGA) (Chernoff et al., 1995), *ade1-6*, *leu2-2*, *his7-1*, *lys9-A21*, *ade2-1* (TAA) (Derkatch et al., 1996). It is difficult to compare the data of various re-

searchers on the specificity of $[PSI^+]$ -mediated suppression due to the differences in the strains used, as well as due to different variants of the $[PSI^+]$ factor.

Concluding remarks

While the main goal of this review was to summarize known data about suppressor mutations isolated in *SUP35* or *SUP45* genes, some other mutations in these genes were not discussed. A number of mutations named PNM (from “[PSI^+] no more”, Doel et al., 1994) have been identified in the *SUP35* gene, affecting the maintenance of the $[PSI^+]$ factor. Such mutations are not known for the *SUP45* gene. A significant amount of antisuppressor mutations was obtained in the *SUP45* gene (Hatin et al., 2007), only some of them are shown in Table 4. We also did not discuss the role of numerous proteins such as ABCE1, Dbp5, PABP, Hrp1, Pub1, Upfs and so on that also take part in translation termination (see Tieg and Krebber, 2013; Schuller and Green, 2018 for recent review).

Multiple effects of mutations in the *SUP35* and *SUP45* genes have been revealed, but their origin still needs to be clarified. It is possible that not only are the *SUP35* and *SUP45* genes involved in translation, but they also participate in the control of other cellular processes. However, an alternative explanation is not improbable: pleiotropic effects may result from a translation termination disruption in the *sup35* and *sup45* mutants. Thus, the story of *sup35* and *sup45* mutations is not finished.

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